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ORIGINAL ARTICLE

The depth of follicular extension in actinic keratosis correlates with the depth of invasion in squamous cell carcinoma: implication for clinical treatment

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Abstract

Background Actinic keratosis (AK) may show extension down follicles, not only in cases with full-thickness epidermal atypia ('bowenoid' AK), but also in cases with atypia limited to the epidermal basalis. Previous studies have demonstrated that, in bowenoid AK, follicular extension is usually superficial, being limited to the upper follicular segment. Little is known about the depth of follicular involvement in cases of invasive squamous cell carcinoma of the skin (iSCC) arising from AK and the role of the follicle in iSCC pathogenesis.

Objective This study investigated the relationship between follicular extension of atypical keratinocytes in an AK and the development of iSCC from the follicular wall. The depth of follicular extension was correlated with the depth invasion of iSCC. Differences between the differentiated and classical pathways of iSCC were also examined.

Methods We performed a retrospective histologic review of 193 biopsy specimens of iSCC with an associated AK. We assessed the presence and depth of follicular extension of atypical keratinocytes in the AK, using tumour (Breslow) thickness and the follicular unit level (infundibular, isthmic and subisthmic), as well as iSCC being present directly adjacent to the follicular basalis.

Results Follicular extension was present in 25.9% of the cases (50 cases), usually extending into the lower follicular segment. The iSCC was present directly adjacent to the follicular basalis in 58% of the cases (29 cases), correlating highly with the depth of follicular extension (infundibular: 3/12; isthmic: 21/33; subisthmic 5/5).

Conclusion The depth of follicular extension of atypical keratinocytes in an AK correlates with the development of depth of invasion of an associated iSCC, irrespective of the pathway of origin. It is therefore important to note the presence and the depth of follicular extension when diagnosing an AK, as follicular extension likely accounts for a significant proportion of recurrent AK and the development of iSCC following superficial treatment modalities.

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Conflicts of interest

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Introduction

Most cases of invasive squamous cell carcinoma of the skin (iSCC) originate from actinic keratoses (AK).[1] Usually, an AK is confined to the interfollicular epidermis, sparing follicular and adnexal epithelium, but in some cases, an AK has extensions of atypical squamocytes down follicular and adnexal epithelium. Follicular extension in an AK may be the precursor of many iSCCs, arising from the follicular basalis.[2,3] Follicular extension

may be present not only in the so-called 'bowenoid' variant of AK, which shows full-thickness epidermal atypia, but also in AK with atypia limited to the basalis.[4] A recent systematic meta-analysis demonstrated that tumour (Breslow) thickness is the characteristic of iSCC most associated with the highest risk of local recurrence and metastasis.[5] As an extension of these observations, we are interested in the significance of follicular extension in AK with respect to the depth of invasion in iSCC.

Interest in follicular involvement in iSCC has been a focus of research and diagnostic interest by numerous researchers.[6–8] Of note, 'follicular' iSCC has been reported to selectively develop in the 'wall' of the hair follicle. Figure 1 shows an example of probable follicular iSCC. It is not clear if these cases represent iSCC associated with an AK, but this case also shows features of a subtle, overlying AK.[9] Subsequent reports have pointed out that follicular iSCC is an underrecognized condition[10,11] Ogawa *et al.*[12] also reported that the acantholytic variant of iSCC often develops from the wall of hair follicle, although not always in the setting of an AK. In addition, there are examples of iSCC which are directly associated with both follicular epithelium and the interfollicular epidermis (Fig. 2).

The understanding of the biology of iSCC is complicated by a lack of common diagnostic criteria and nomenclature. First, there are no clear-cut criteria to differentiate an early AK from chronic actinic damage, leading to different histopathological interpretations of the same entity. Second, some reports appear to use squamous cell carcinoma *in situ*, commonly termed 'Bowen's disease,' when diagnosing a bowenoid AK or HPV-associated genital neoplasia, both which are entirely different clinicopathological entities with different immunohistochemical profiles. This diagnostic confusion is complicated by the observation that both Bowen's disease and bowenoid AK may show follicular extension of atypical keratinocytes.[13]

This study aims to demonstrate that follicular extension of an AK is associated with the depth of invasion (Breslow) of iSCC.

Materials and methods

Biopsy cases of iSCC from sun-exposed areas diagnosed during three consecutive years were retrieved from the files of the Department of Anatomic Pathology at the Hospital Universitari Germans Trias i Pujol, Badalona, Spain. We excluded all biopsy specimens smaller than 3 mm and larger than 2.5 cm in size and those specimens with poor architectural preservation (i.e. superficial or fragmented specimens and tumours with extensive

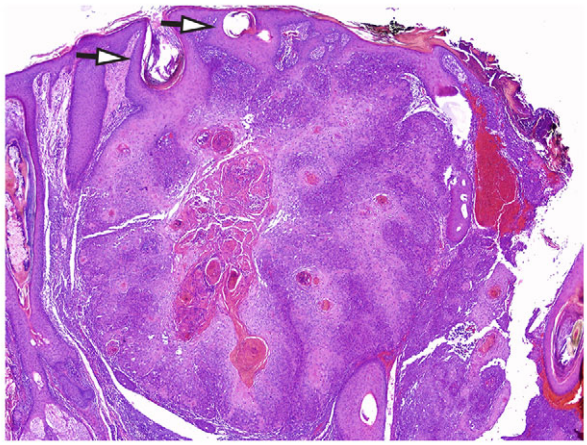


Figure 2 Invasive squamous cell carcinoma directly attached to follicular epithelium and the interfollicular epidermal basalis. Two partially preserved infundibula are indicated by arrows.

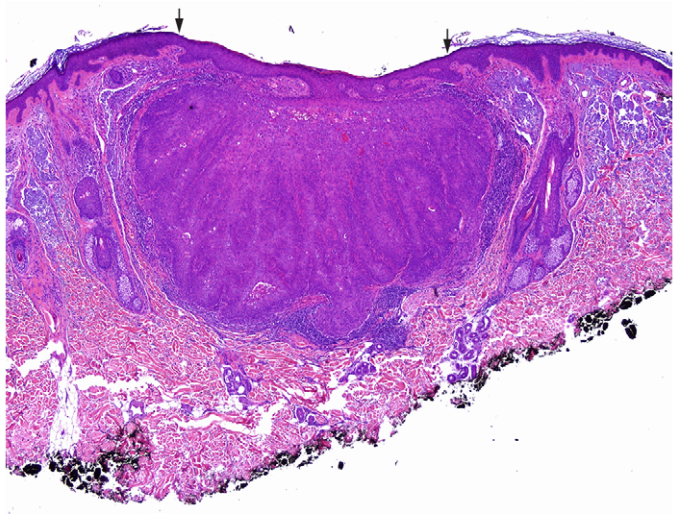


Figure 1 An example of so-called 'follicular' invasive squamous cell carcinoma set in an area with severe actinic damage. The epidermis overlying the tumour (delimitated by arrows) shows irregular some enlarged keratinocytes, especially along the basalis with extension to the follicular isthmus and subtle with parakeratosis.

ulceration). Cases of Bowen's disease[14,15] HPV-associated iSCC and infundibulocystic iSCC[16] were also excluded.

All H&E sections were studied by three dermatopathologists (MTFF, XS and PV), searching for follicular extension of atypical keratinocytes along the follicular basalis and near the iSCC. The depth reached by the atypical keratinocytes was measured in millimetres from the epidermal surface (Breslow thickness) and also associated with the level of the follicular unit, using the sebaceous gland as a landmark. Follicular extension was classified as 'infundibular' when atypical keratinocytes were located above the sebaceous gland; 'isthmic' when atypical keratinocytes involved the sebaceous duct and/or sebaceous gland; and 'subisthmic' when atypical keratinocytes were present below the sebaceous gland (Fig. 3). Involvement of the eccrine sweat glands was also analysed.

As one additional assessment, the tumours were classified based upon the location and the extent of the atypical keratinocytes in the overlying interfollicular tumour. Nomenclature which has been applied to epithelial tumorigenesis in the skin and other anatomic sites (anogenital and oral) was employed.[17–19] Cases in which the AK consisted of atypical keratinocytes confined to the lower one-third of the epidermis near the basalis were classified as having originated through the differentiated pathway. Cases in which the AK shows near full-thickness atypia were classified as having originated through the classical pathway.

Statistical study

Statistical analysis was performed using SPSS (V11.5) software (SPSS Inc., Chicago, Il, USA), with two-sided tests. The statistical significance of differences in depth of follicular invasion was evaluated using the Mann–Whitney test and Kruskal–Wallis test, as required. Fisher's exact test was used to determine the statistical significance of differences between proportions. Statistical significance was set at $P < 0.05$.

Results

We identified 503 cases of iSCC diagnosed over a period of 3 years, and 193 of them fulfilled the criteria for the study. Follicular extension of atypical squamocytes in an AK was identified in 50 cases (25.9%). Of the 50 cases with follicular extension, 12 cases reached the infundibular level, 33 reached the isthmic level and five reached the subisthmic region. iSCC originated from the follicular basalis in three of 12 cases with infundibular involvement (25%), 21 of 33 cases with isthmic involvement (63.6%) and five of five cases with subisthmic involvement (100%; $P < 0.01$). The median tumour thickness of iSCC developing from the follicular basalis was higher than in iSCC arising from the epidermis (1.75 mm vs. 0.9 mm, $P < 0.001$).

In assessment of the characteristics of the tumour surface, 122 cases were classified as having originated from the 'differentiated pathway' and 71 cases with near full-thickness atypia were considered to have originated through the 'classical pathway'. The

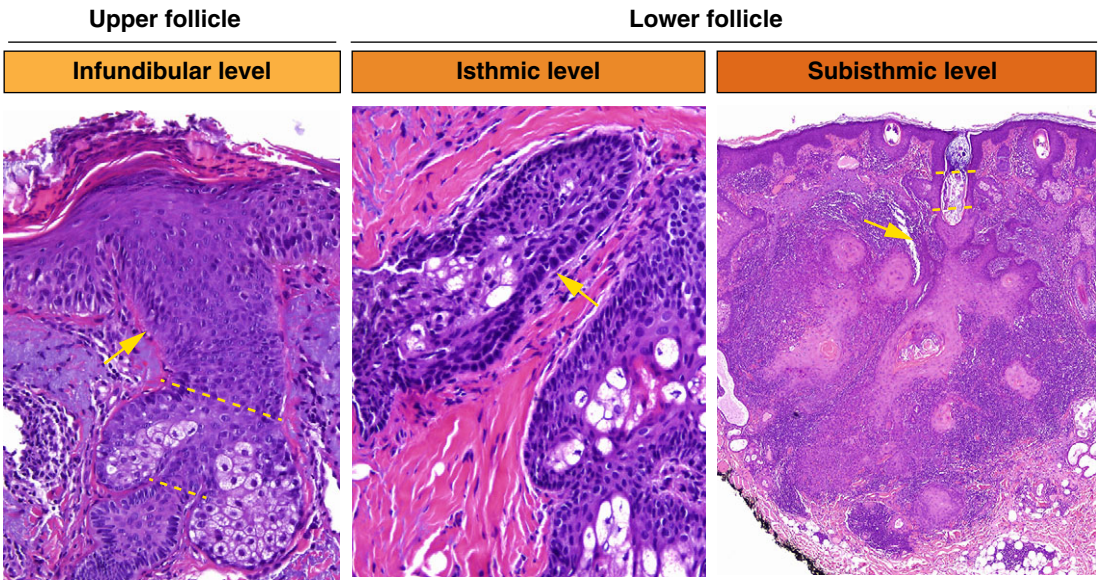
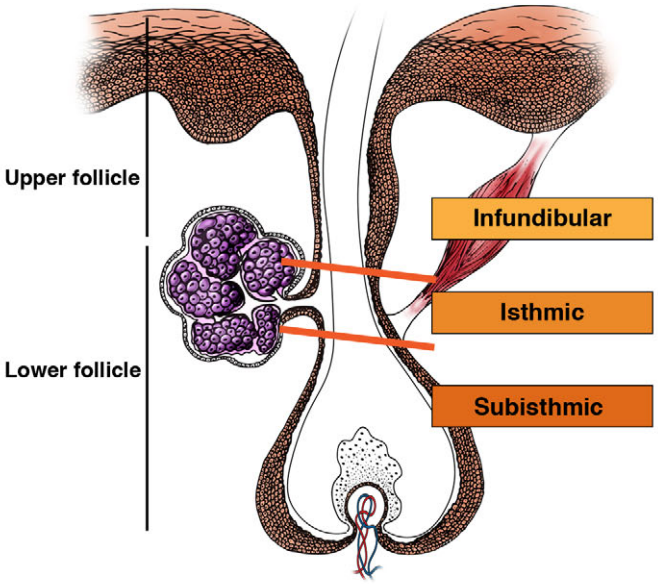


Figure 3 The depth of follicular involvement was classified into three levels using the sebaceous glands as a guide as follows: infundibular level when the atypical cells reached only the epithelium located above the sebaceous glands; isthmic, when the atypia involved the sebaceous ducts and/or sebaceous glands; and subisthmic, when the atypical cells where present below the sebaceous glands. Follicular levels are delimitated with dotted lines. Arrows indicate involvement by atypical cells that in the subisthmic segment is associated with invasive squamous cell carcinoma.



Atypical cells in the hair follicles $n = 50$	iSCC originated from the follicular basalis $n = 29$
12 cases	3 cases (25%)
33 cases	21 cases (63.6%)
5 cases	5 cases (100%)

Figure 4 The three follicular levels (infundibular, isthmic and subisthmic) are delimited with dotted lines. Follicular involvement by atypical cells was observed in 50 of the 193 studied cases. In 12 of them, the atypia reached the upper portion of the follicle (infundibulum), and in three of them (25%), there was evidence of invasive squamous cell carcinoma (iSCC) originated from the follicular basalis. In 33 cases, the atypical cells reached the isthmic portion, and in 21 of them (63.6%), iSCC originated from the follicular basalis. In five cases, the atypical cells reached the isthmic portion and in all of them iSCC originated from the follicular basalis.

proportion of those corresponding to the differentiated pathway (36/122, or 29.5%) was higher than that corresponding to the classical pathway (14/71, or 19.7%), but the difference was not statistically significant. No significant differences in depth of follicular involvement were found between iSCC originating through the differentiated and the classical pathways.

For eccrine sweat gland involvement, atypical keratinocytes were limited to the acrosyringia in 39 cases, but the atypical keratinocytes never extended to the dermal level of the duct and no iSCC appeared to originate from the sweat gland epithelium.

Discussion

This study demonstrates that the depth of follicular extension of atypical squamocytes in an AK directly correlates with the depth of invasion of an associated iSCC. The study complements a prior study which showed only superficial follicular extension in bowenoid AK[13] Taken together, the findings strongly suggest that deeply invasive SCC often arises from the follicular basalis.

This study has direct therapeutic implications, providing an explanation for recurrence and for progression of some AKs following superficial destructive treatment modalities, such as cryotherapy, which are unlikely to reach the deeper levels of the follicular epithelium. If the histopathological assessment carefully notes the presence and the depth of follicular extension of an AK, then more aggressive treatment modalities, such as curettage, excision or the use of photosensitizers, may be employed. Such treatment would likely limit the incidence of recurrence and the development of iSCC.

The relationship between the atypical squamocytes of the AK and the malignant squamocytes of the iSCC with follicular stem cells in the bulge is an additional point of interest. Studies in nude mice have demonstrated that malignant squamous cells may take advantage of the follicular cycle machinery to overcome negative regulatory signals and induce growth.[8] Disruption of an anagen phase follicle by follicular extension could induce follicular cycling into catagen phase. When the follicle then cycles back into anagen phase, hair follicle stem cells are released from PTEN-induced quiescence, being able to trigger an intense epithelial proliferation during the early anagen phase.[8] Mutated keratinocytes may also respond and proliferate, leading to iSCC.[6,7] Even though the results of these animal experimental studies may be difficult to translate to humans, our work supports the idea that the hair follicle may contribute significantly to the development of deeply iSCC. Initiation of dermal invasion at a deeper level likely leads to a more aggressive iSCC than iSCC arising from the epidermis.

Of particular interest, this study suggests that the sweat gland does not play a major role in the development of iSCC in the presence of an AK. In this study, the sweat glands were rarely involved, only in their most superficial portion, and iSCC was

never identified in their proximity. These observations reinforce the concept that only the hair follicle, among adnexal structures, plays a major role in the genesis of iSCC.

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